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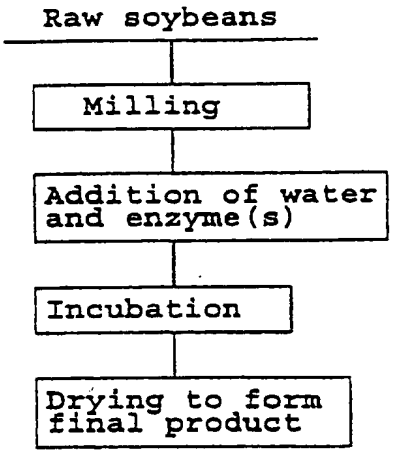
(54) Title: A PROCESS FOR PRODUCING A NUTRITIONAL PRODUCT AND PRODUCTS PRODUCED

(57) Abstract

The present invention relates to the production of nutritional products from oilseeds, especially from soybeans by an enzymatic treatment. Water is added to an oilseed material which has been obtained by mechanical disruption of whole raw oilseeds. The resulting aqueous mixture will include substantially the entire fibrous and proteinaceous components of said seeds and the oilseed proteins will be in a substantially non–denatured condition. A proteolytic enzyme is added and said aqueous mixture is incubated for a time sufficient to cause degradation of oilseed proteins and any antinutritional factors. In the raw and non–denatured oilseed material the solubility and degradability of the proteins is enhanced and the antinutritional factors are destroyed without any heat treatment. The invention also relates to nutritional products produced by the enzymatic treatment of oilseeds, and especially to fish feed obtained from soybeans.



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A process for producing a nutritional product and products produced

The present invention relates to the production of nutritional products from oilseeds, especially from soybeans by an enzymatic treatment. The invention also relates to nutritional products produced by the enzymatic treatment of oilseeds, and especially to fish feed obtained from soybeans.

Oilseeds such as soybean, rapeseed, cocoanut, sunflower, peanut, etc. are generally processed in various ways to obtain a high yield of oil from the seeds. However, it has been recognized that the proteins of the oilseeds form an important and a cheap source of protein. Thus, oilseed materials have also been processed for recovering the proteins in conjunction with recovering the oil.

The nutritional value of many oilseed proteins such as soy protein is, however, reduced by antinutritional factors such as trypsin inhibitors. The standard soy meal processes include solvent treatment and heating operations which reduce the activity of the antinutritional factors, but these operations also cause denaturation of the proteins thereby decreasing the solubility and digestability of said proteins. In addition to the protein denaturation, the standard operations cause covalent or non-covalent fixation of the proteins to surrounding carbohydrate structures. This further decreases the solubility and digestability of the proteins.

Several processes are known for enzymatically decomposing oilseed materials either to improve the oil yield or to improve the protein yield.

A review of aqueous and enzymatic processes for oil extraction from oilseeds made by Rosenthal A., et al. in Enzyme and Microbial Technology 19:402-420, 1996 makes evident that enzymatic treatment of oilseed materials increases the oil yield. However, little is said about increasing the protein yield.

US Patent 3,640,725 describes a process for preparing nutritional products from whole soybeans and other oilseeds by incubating ground oilseed material in water with a proteolytic enzyme. Prior to the enzyme treatment the material is subjected to a heat treatment at about 120°C in order to inactivate the trypsin inhibitor and to break up the protein structure.

US Patent 3,640,723 describes a process for preparing animal fodder by treating soy meal in an aqueous suspension with pectinase and cellulase to produce an easily digestible product having an increased fraction of soluble reducible sugars. The process for obtaining the soya meal used as raw material obviously includes oil removal by standard methods denaturating the proteins.

US Patent 4,324,805 describes a process for producing a soy protein hydrolysate from a fat-containing soy material by washing full fat soy flour with water at a pH of 4-4.5 until all soluble material is removed. The washed solid is subjected to hydrolysis by a proteolytic enzyme at pH 7.5-8.

WO 95/29598 describes an enzymatic treatment of soy suspensions aiming at reducing their viscosity. The described treatment includes a heat treatment step in order to inactivate the trypsin inhibitor.

EP 0 199 981 relates to a soybean hydrolysate based on the entire constituents of the beans. A slurry of pulverized whole soybeans is mixed with water at 80°C and then homogenized in a high pressure homogenizer. The homogenate is then hydrolysed with a protease.

US Patent 4,100,024 describes a process for the hydrolysis of a soy isolate by proteolytic enzymes. The hydrolysis is halted before a too high degree of hydrolysis has taken place to prevent the production of bitter tasting low molecular peptides.

GB 1,107,229 discloses a process for producing protein hydrolysates by an action of a proteolytic enzyme and a phosphatase. The protein, such as lactalbumin, should preferably be in an undenatured condition. According to the Patent the process is suitable also for soybean meal but no examples of such a process are given.

Many of the background art oilseed processes aim primarily at recovering the oil either by solvent or aqueous extraction or by pressing. The oilseed proteins are often damaged or denatured in the oil recovery process.

The prior art protein recovery processes also include complicated and/or energy consuming processing steps which in one way or another cause denaturation and irreversible aggregation of the proteins within the fibrous carbohydrate structures. An important step also includes removal of soluble carbohydrates. These process steps while

being considered necessary for the inactivation of antinutritional factors present in the oilseed and/or for hydrolyzing the proteins, in fact, also counteract a proper degradation of the proteins. Especially, the aggregation of the proteins decreases the rate of digestion process thereby retarding animal's nutrient uptake processes.

Accordingly, there exists a need for improving the bioavailability of proteins from oilseeds such as soybeans.

An object of the present invention is therefore to provide a process for the recovery of nutritional components from oilseeds, especially from soybeans in a technically simple way.

Another object is to provide an enzymatic process which has low energy consumption.

A further object of the invention is to obtain the oilseed proteins in an easily digestible form without antinutritional factors.

An object of the invention is also to provide an animal feed based on oilseed components wherein a large proportion of the oilseed components is used for nutritional purposes.

A further object of the invention is to provide a fish feed from oilseeds, especially from soybeans.

The present invention is based on the realization that raw non-denatured oilseed material can be treated in such a way that the antinutritional factors are destroyed without any heat treatment. The use of raw and un-dried oilseeds enhances the water solubility and degradability of the oilseed proteins in comparison to the standard procedures. Proteolytic degradation of the essentially non-denatured proteins of such raw oilseeds provides a very homogeneous protein fragmentation. The polypeptide chains have a small and uniform size compared to degradation products formed by conventional methods. The proteinaceous antinutritional factors will also be degraded and there is no need for the prior art process of toasting to inhibit the action of said inhibitors.

The present invention is defined in the appended claims. Thus, the present invention concerns a process for producing a nutritional product from oilseeds comprising the steps of

a) adding water to an oilseed material obtained by mechanical disruption of whole,

optionally dehulled raw oilseeds to provide an aqueous mixture of said oilseed material, said mixture including substantially the entire fibrous and proteinaceous components of said seeds and having the oilseed proteins in a substantially non-denatured condition;
b) adding a proteolytic enzyme capable of degrading oilseed proteins including proteinaceous antinutritional factors in said aqueous mixture;
c) incubating said mixture at a temperature below the inactivation temperature of said enzyme for a time sufficient to cause degradation of said oilseed proteins and antinutritional factors; and
d) at need, concentrating and/or drying the resulting product or a portion thereof.

The whole of the treatment preceding or encompassed in step a) of the process should be performed at non-denaturating conditions so as to avoid denaturation and aggregation of the proteins. The preferred oilseeds are soybeans, although the process is suitable for the treatment also of other oilseeds with similar characteristics.

By the term "raw" oilseed material as used herein is meant any form of oilseed, such as soybeans which have not been treated in such a way that the native proteins have lost their native conformation and become wholly or substantially denatured. Said term especially means that the seeds have not been subjected to solvent extraction, heat treatment (toasting), acid treatment etc., which treatments are conventionally used to extract oil and/or to inactivate the antinutritional factors and/or to remove soluble carbohydrates and/or to strongly denature the proteins. The term is not intended to exclude mild treatments of the seeds or seed material which treatments do not substantially affect the subsequent hydrolysis of the proteins. It is essential for the present invention that in the mixture of water and oilseed material the proteins, including any proteinaceous antinutritional factors are substantially non-denatured.

The "raw" oilseed material may exist in any physical form which is derived from raw whole oilseeds, which may or may not have the oil removed by non-denaturating methods, such as by pre-pressing.

The term "raw" oilseed as used herein should also be taken to indicate that the seeds are essentailly un-dried, i.e. they have not been subjected to any stringent drying process. It is indeed critical to the present process that the proteins of the seeds should not have become unduly aggregated or attached to the fibrous carbohydrate structures due to a drying and/or heating treatment.

The term "raw" oilseed does not, however, exclude normal drying of the seeds in order to remove excess moisture which would provide a risk for mold growth during shipping. Thus, the oilseeds would normally be in the raw condition in which they are shipped.

It should be noted that although heating and drying are detrimental to the present process, the whole raw oil seeds may have been subjected to mild water treatments, such as soaking for softening or even germination, prior to being mechanically disrupted. Any soaking should, of course be performed in such a way as to avoid dissolving any substantial portion of the seeds.

The raw oilseeds may be dehulled prior to crushing although the hulls may be included in the mass to be treated. The purpose of the crushing operation is mainly to break the seed structure in order to expose the proteins within the fibrous structure to the action of water and enzymes. The term "crushing" should be considered to include other mechanical operations such as milling, grinding or flaking which break the seed structure.

The crushing preferably includes pressing to release at least part of the oil. The oil may be recovered separately or it may be left in the material to form part of the final product.

The crushed oilseeds and water should preferably be agitated or mechanically mixed to provide a mixture or a slurry to rapidly obtain an intimate contact between water and solid material. The aim is to solubilize a major portion of the proteins, including the antinutritional factors into the aqueous medium. Since the oilseed material has not been dried and/or heated, the water will be able to penetrate through the carbohydrate network surrounding the discrete cellular organelles storing the proteins. As the proteins are not denatured, aggregated or completely dried, they will be rapidly solubilized and thereafter they will be able to diffuse with the water out of the plant matrix.

Even though some protein remains within the plant matrix this protein material will be reached by the digestive enzymes since the enzyme will be able to penetrate into the fibrous matrix due to its non-aggregated condition.

The peptide composition of oilseeds such as soybeans differs from animal based protein sources, such as fish meal or meat meal. Soy protein sources have less than ten major polypeptide components whereas animal based sources have hundreds of polypeptide components. Thus, the digestion rate differences of individual polypeptides have greater effect on the total degradation rate in soy protein sources. Furthermore, the animal based

protein sources contain more fragmented polypeptides than soy protein sources which makes them easily digestible.

A problem with the prior art processes of digesting soy proteins is that there is a tendency for the digestion to proceed "unevenly" splitting some proteins into amino acids or small peptides while leaving other proteins in intact form or as partially hydrolyzed polypeptide fragments. The present process causes the protein source to be uniformly digested to provide a product wherein a major proportion of the polypeptides have been hydrolyzed to small-sized polypeptides, oligopeptides or even free amino acids. Said uniformly digested product shares more similarities with animal based protein sources, such as fish meal and meat meal, than the products of the prior art processes.

The preferred proteolytic enzymes or mixtures of enzymes to be used in the present process have broad substrate specificity in order to hydrolyze the substrate proteins from multiple sites. Said enzymes or mixtures of enzymes are often derived from Bacillus spp.

According to a preferred embodiment of the process, one or more additional enzymes are added having similar or different degrading activity as said proteolytic enzyme and being compatible with said proteolytic enzyme. Said additional enzymes may be selected among hydrolytic enzymes, cellulolytic enzymes, hemicellulolytic enzymes, phosphorolytic enzymes and proteolytic enzymes, and mixtures thereof. A typical useful phosphorolytic enzyme is phytase.

In the preferred embodiment of the process, the water and proteolytic enzyme are added simultaneously to the crushed oilseeds. This may be done, for instance, by spraying an aqueous enzyme solution on the crushed seeds and mixing to provide a slurry. If desired, however, the water may be added first to provide a mixture wherein the temperature and pH are adjusted to a level suitable for the selected enzyme(s).

For the degradation to proceed smoothly, the pH of the aqueous mixture should be adjusted to a level which is at or close to the optimum activity pH of the active enzyme(s). The optimum pH will naturally depend on the selected enzyme, but it has been found that a pH of 7.0 to 8.0 is suitable to the preferred proteolytic enzymes.

As the degradation proceeds, the pH decreases and should be adjusted in the conventional way by the addition of suitable bases such as sodium hydroxide. As an alternative and advantageous procedure, the present invention encompasses the use of different enzymes

having different pH optima. Thus, as the pH decreases, a different enzyme will be added having its optimum activity at a lower pH than the previously added enzyme. As the pH continues to decrease, new enzymes may be added having their optimum activities at even lower pH values. Alternatively, all enzymes may be added together at the beginning of the process. In such a case the enzymes having lower pH optima should be resistant to the activity of the enzymes having higher pH optima. In this way an adjustment of the pH will not be necessary and the product will be digested by different enzymes having different activities. Said multi-enzyme system may be constructed from different *Bacillus* proteases and it may include, for example, an alkaline protease and a neutral protease.

The water which is added to the crushed oilseeds should be at a temperature below the denaturating temperature of the proteins, preferably between 15 and 75°C, more preferably at about 30 to 50°C. However, some proteins may denature even at temperatures below 55°C. If denaturation of the dissolved proteins takes place in solution, it need not negatively affect the rate of proteolysis. Therefore, partial denaturation during the enzyme treatment is not necessarily unacceptable to the process, but excessive denaturation leading to aggregation and retarded diffusion of the proteins has to be avoided. The water is preferably added at a temperature at or close to the temperature at which the proteolytic enzyme has its optimum activity.

The incubation conditions, especially pH and temperature should be controlled to provide a desired degree of degradation and reduction of size of the proteins. The incubation is generally performed at 20-50°C, preferably at 30-50°C for a time sufficient to provide a desired degree of degradation.

The objective of the protein hydrolysis is to provide small peptides, and the hydrolysis can be followed indirectly by taking samples during the incubation, extracting them under highly denaturing conditions and analyzing the formed extract with gel electrophoresis. The appearance of native-sized polypeptides or large polypeptide fragments are indications of incomplete hydrolysis.

The present invention further provides a nutritional oilseed product, especially a soy protein product, which comprises the degradation products of substantially non-denatured oilseed proteins, including the degradation products of proteinaceous antinutritional factors, derived from raw oilseeds, said proteins having been degraded by the action of proteolytic enzymes in an aqueous mixture.

The present invention also concerns an animal feed or feed additive based on oilseed protein, especially soy protein, which comprises the proteolytic degradation products of aqueous non-denatured oilseed proteins and proteinaceous antinutritional factors of raw oilseeds, especially raw and undried soy beans.

The proteins in the products according to the invention are degraded in a uniform manner, said degraded protein molecules having a size distribution wherein 80 % are less than about 10 kDa measured by gel electrophoresis. Preferably up to 90% or more of the molecules are less than about 10 kDa.

In a special preferred embodiment, the present invention concerns a fish feed which consists essentially of proteolytic degradation products obtained by degrading substantially non-denatured soy proteins in an aqueous mixture of crushed raw soybeans. The degradation product should not contain significant amounts of large polypeptides and at least 80-90% of the molecules should be below 10 kDa.

The nutritional products optionally also contain oil, such about 5 to 35 % or more of the initial oil content of the seed.

The invention will now be described in greater detail referring also to the accompanying drawing, wherein

Fig. 1 shows a block diagram of a very simple embodiment of the invention, and

Fig. 2 shows a block diagram of an embodiment for obtaining a highly hydrolyzed protein product accorsing to the invention.

The detailed description is mostly made with reference to the preferred oilseeds, i.e. soybeans and soy protein. The invention is, however, not limited only to the preferred embodiments disclosed in the detailed description. A person skilled in the art will understand that the invention may be varied in many ways without deviating from the general scope of the claims.

According to the invention, the crushed fresh raw soy material is mixed with water to provide an aqueous mixture or slurry. The oil released at crushing may be removed, if desired, or it may be retained in the mixture, if the oil is to form part of the final product. The oil may also be removed at a later stage of the processing.

Optionally a suitable buffer is added to the water or to the mixture. The proteolytic enzyme(s) may be added simultaneously with the water or it/they may be added after the addition of water to allow time for at least a portion of the proteins to dissolve. The ratio between liquid and raw soy material may vary depending on the application and on the later processing operations.

The four most important parameters for the proteolytic enzyme are the following. Firstly, the proteolytic enzyme should not be inhibited by any compound present in raw soy material, especially it should not be inactivated by soy bean trypsin inhibitors. Secondly, the proteolytic enzyme should be capable of efficiently degrading and thereby inactivating the antinutritional factors, such as trypsin inhibitors, lectins and allergens present in the soybean material. Thirdly, the proteolytic enzyme should have a broad specificity and it should be capable of efficiently degrading all major soy proteins in addition to the proteinaceous antinutritional factors. Fourthly, the proteolytic enzyme should have a high enough activity in order to degrade raw soy material under a reasonable time scale.

The proteolytic activity may be provided by any pure protease or mixture of different proteases or other enzymes which fulfill the above mentioned properties. Good results have been obtained with enzymes of *Bacillus* sp., especially with alkaline protease derivative and with neutral protease derivative. However, suitable enzymes are obtainable also from other protease producing sources, such as *Aspergillus*, even plant protein sources.

Said enzymes may advantageously be improved by e.g. recombinant techniques. A person skilled in the art will be able to select suitable enzymes, e.g. from among a variety of known and commercially available products.

The working temperature and the incubation time depend on the amount of added proteolytic enzyme and its properties. Preferably, the process is carried out at 25-50 °C for less than one day with a proteolytic activity which is between 100 and 10 000 proteolytic activity units per gram of raw soy material. Higher incubation temperatures normally cause faster inactivation of the enzyme while lower incubation temperatures require higher enzyme doses or longer incubation times.

In the practice of the present invention the pH of the raw soy material is initially set near the pH optimum of the proteolytic enzyme. Proteolysis causes a decrease of the initially

set pH and reduces the rate of proteolysis. This drop in the rate of proteolysis can be prevented by two different means. The pH of the raw soy material slurry may be continuously monitored and adjusted by alkaline addition. However, instead of adjusting the pH, a mixture of proteases having different pH optima can be used. Thus, the pH reduction inactivates one enzyme and simultaneously activates another enzyme having a lower pH optimum. The latter method does require that the different proteolytic enzymes are resistant to each others activity or the later enzymes may be added at a later stage when the optimum rate of the first enzyme has passed.

For some applications, other than proteinaceous antinutritional factors should be removed from the soy material. The process of the present invention can be used for enzymatically removing also non-proteinaceous antinutritional factor. For example, phytate or oligosaccharides which are present in raw soy material can be degraded with suitable enzymes. The requirement for the non-proteolytic enzymes is that they are resistant to the degrading activity of the proteolytic enzymes and are not inactivated thereby. Said resistant enzyme systems can be found from different microbial fermentation products, especially from products derived from Bacillus spp, where the enzymes have become resistant against each other's activities during the course of evolution.

The protease treated raw soy material can be processed in two different ways depending on the intended use or application of the product. The treated material may be dried, preferably by using methods based on water evaporation. These methods leave an insoluble material, such as soy fiber, in the final product.

Alternatively, the treated material may be pressed, filtered, extracted or centrifuged in order to separate the soluble and insoluble parts of the material. The water soluble fraction contains the most valuable nutrients. Any oil remaining in the liquid phase may be separately recovered, or it may be left in the product to serve as an additional source of nutrition.

If desired, the aqueous degradation products may be absorbed in an edible or non-toxic water insoluble solid such as into a fraction of crushed dehulled soybeans in order to save the energy required to dry the product and in order to avoid high-cost drying process such as spray-drying or freeze-drying, and to replace them with low-cost drying processes, such as drum-drying.

Fig. 1 of the drawing indicates a very simple and energy saving procedure for obtaining a

hydrolyzed oilseed product. An essential feature of the invention is that the oilseeds to be used are raw or fresh. Thus, they should not be extensively dried, nor should they have been subjected to any denaturing procedure before being used in the present process.

The whole raw seeds are milled, ground or crushed to provide a mixture of broken hulls and seed material. The dry substance of a soybean, for instance is made up of about 8 % hull, 2 % hypocotyl and about 90 % cotyledon. The cotyledon contains in turn fat, carbohydrates, fibres, proteins and ash material. The proportion of protein is about 45 % of the total cotyledon material.

The milling breaks up the walls surrounding the fat and protein and exposes the same to the action of water and enzymes which is simply sprayed onto the milled mass of seeds. A certain mixing of the mixture is required in order to allow the water and enzymes to contact the proteins in a slurry.

The seeds are not subjected to any toasting or extraction or washing procedures. The temperature of the water and the pH is adjusted to suit the enzyme and the slurry is incubated for a sufficient time to allow a substantial hydrolysis to take place. The rate of hydrolysis can be monitored by analyzing the product with gel electrophoresis. When no large protein fragments remain and a desired level of hydrolysis has taken place, the slurry may, if desired, be dried in any suitable way.

The product contains the oilseed proteins in a hydrolyzed and easily digestible form. The toxic proteinaceous antinutritional factors are also degraded. The carbohydrates and fibers may be intact or they may have been degraded also by enzymes. The product is suitable as animal feed, for instance, for pigs.

Fig. 2 shows a more sophisticated and preferred process according to the invention. Here raw soybeans are crushed by pre-pressing to release the soya oil. The oil is removed in the conventional way which is well known to those skilled in the art.

Water at a non-denaturing temperature is added to the crushed beans and the mixture is agitated to make a slurry. The pH of the slurry is adjusted at need to meet the demands of the proteolytic enzyme which is added to the slurry. The slurry is incubated for a time sufficient to degrade the proteins until no large polypeptide chains remain.

The insoluble material is thereafter separated from the slurry. If desired, some or all of the

freed oil may be recovered at this stage. The soluble fractions concentrated and either mixed into an edible carrier or dried to produce a final protein product. The product contains no proteinaceous antinutritional factors. It is easily digestible and it resembles in character the fragmentation of fish meal. It may be used as a substitute for fish meal in the feeding of fish and other animals.

Fish feed for salmon and trout should not contain any of the insoluble portions of the soybeans, but may preferably contain at least some of the oil. Other fish, such as carps may be able to eat also feed containing a minor portion of the insoluble fraction.

The process may also produce a nutritional protein product for human or animal use. The product comprises the proteolytic degradation products of non-denatured soya proteins. The antinutritional factors have also been degraded making the soy material non-toxic.

The soy proteins have been degraded to substantially uniform small fragments so that at least 80% thereof, preferably at least 90% thereof have a size less than 10 kD. No large antigenic polypeptides remain in the product. The product may contain more or less of the initial soya oil, depending on the intended use.

The invention will now be illustrated by means of a few non-limiting examples.

Example 1

A 5g sample of raw soy beans was subjected to pre-pressing to release the oil. The oil was subsequently removed, and the crushed beans were combined with 20g of water to provide a slurry. The slurry was incubated at 20 °C for one hour and then centrifuged at 31 000 g for 15 minutes.

The resulting supernatant fraction contained about 0.16g protein/g DM. The protein concentration was adjusted to 10mg/ml by addition of water and an enzyme preparation having proteolytic activity derived from *Bacillus* species was added (P 3000, produced by Genencor Inc.).

The proteolytic treatment was performed at four different enzyme levels, i.e. at 0, 100, 1 000 and 10 000 U/ml. The pH of the mixture was about 6.8. The mixture was incubated 1 hour at 37 °C.
The protease treated mixture was shown to have a diminished concentration of soy bean

lectin when 100 U/ml dose was used. The mixture was shown to be totally lectin free when 10 000 U/ml was used. The lectin concentration was measured by using Western blotting technique and anti-soy bean lectin antibodies.

SDS-PAGE method was used to demonstrate the protease treated mixture contained highly degraded soy bean polypeptides, and that no Kunitz-type or Bowman-Birk-type protease inhibitor were visible.

Example 2

A 5 g sample of raw and pre-pressed soy beans was combined with 20g of water to form a slurry and an enzyme preparation having a proteolytic activity was added to reach a final activity of 0, 4 000 and 40 000 U/g DM. The added enzyme preparation was derived from *Bacillus* species.

The slurry was incubated at 37 °C under moderate agitation. After 1.5 hours the slurry was centrifuged at 31 000 g for 15 minutes.

The resulting supernatant was shown to be lectin free using Western blotting technique and anti-soy bean lectin antibodies. In addition, SDS-PAGE method showed the resulting supernatant contained highly degraded soy bean polypeptides, and no Kunitz-type or Bowman-Birk-type protease inhibitor were visible.

Example 3

A 5g sample of raw milled soy beans were combined with 20g of water and an enzyme preparation having proteolytic activity was added to reach a final activity of 0, 4 000 and 40 000 U/g DM. The added enzyme preparation was derived from *Bacillus* species.

The slurry was incubated at 37 °C under moderate agitation. After 1.5 hours the slurry was centrifuged at 31 000 g for 15 minutes.

The resulting supernatant was shown to be lectin free using Western blotting technique and anti-soy bean lectin antibodies. In addition, SDS-PAGE method showed the resulting supernatant contained highly degraded soy bean polypeptides, and no Kunitz-type or Bowman-Birk-type protease inhibitor were visible.

Claims

1. A process for producing a nutritional product from oilseeds, comprising the steps of
a) adding water to an oilseed material obtained by mechanical disruption of whole, optionally dehulled raw oilseeds to provide an aqueous mixture of said oilseed material, said mixture including substantially the entire fibrous and proteinaceous components of said seeds and having the oilseed proteins in a substantially non-denatured condition;
b) adding a proteolytic enzyme capable of degrading oilseed proteins including proteinaceous antinutritional factors in said aqueous mixture;
c) incubating said mixture at a temperature below the inactivation temperature of said enzyme for a time sufficient to cause degradation of said oilseed proteins and antinutritional factors; and
d) at need, concentrating and/or drying the resulting product or a portion thereof.

2. Process according to claim 1, c h a r a c t e r i z e d in that said oilseed proteins in said mixture of step a) are in a totally non-denatured condition.

3. Process according to claim 1 or 2, c h a r a c t e r i z e d in that said oilseeds are raw soybeans.

4. Process according to claim 1, 2 or 3, c h a r a c t e r i z e d in that said mechanical disruption of said oilseeds includes pressing to release oil at least a portion of said oil being removed prior to step a), said procedure being performed while maintaining non-denaturating and non-aggregating conditions.

5. Process according to claim 1, c h a r a c t e r i z e d in that said water is added at a temperature below the denaturating temperature of the oilseed proteins, preferably 15 and 75 °C, more preferably at about 30 to 50 °C

6. Process according to claim 5, c h a r a c t e r i z e d in that said water is added simultaneously with said enzyme at a temperature at or close to the temperature at which said proteolytic enzyme has its optimum activity.

7. Process according to claim 1, c h a r a c t e r i z e d in that the pH of said water is adjusted to a level at or close to the optimum activity pH of said proteolytic enzyme.

8. Process according to claim 1, c h a r a c t e r i z e d in that said proteolytic enzyme is an enzyme derived from Bacillus sp.

9. Process according to claim 1, characterized in that said process further comprises adding one or more additional enzymes having similar or different degrading activity as said proteolytic enzyme and being compatible with said proteolytic enzyme.

10. Process according to claim 9, characterized in that said additional enzymes are selected from the group consisting of hydrolytic enzymes, cellulolytic enzymes, hemicellulolytic enzymes, phosphorolytic enzymes and proteolytic enzymes, and mixtures thereof.

11. Process according to claim 9, characterized in that said additional enzyme is a proteolytic enzyme, the optimum activity of which is at a lower pH than the one of said first proteolytic enzyme.

12. Process according to claim 1, characterized in incubating said mixture while controlling the degradation conditions to provide a desired degree of degradation and reduction of size of said proteins substantially to below 10 kDa.

13. Process according to claim 1, characterized in separating said incubated mixture into a solid phase and a liquid phase, and removing said solid phase.

14. Process according to claim 13, characterized in recovering oil from said liquid phase.

15. Process according to claim 13 or 14, characterized in absorbing said liquid phase, preferably after concentration thereof, in an edible carrier, and optionally granulating the same to provide an animal feed.

16. Process according to claim 15, characterized in that said edible carrier comprises said solid phase.

17. A nutritional oilseed protein product, characterized in that it comprises the proteolytic degradation products of substantially non-denatured soya proteins, including proteolytically degraded proteinaceous antinutritional factors, said proteins having been degraded by the action of proteolytic enzymes on raw oilseeds in an aqueous mixture.

18. A nutritional oilseed protein product, according to claim 17, wherein said proteins are soy proteins which have been degraded to substantially uniform small fragments so that at

least 80% thereof, preferably at least 90% thereof have a size less than 10 kD.

19. Animal feed or feed additive based on oilseed protein, c h a r a c t e r i z e d in that it comprises the proteolytic degradation products of aqueous substantially non-denatured oilseed proteins and proteinaceous antinutritional factors of raw oilseeds.

20. Animal feed or feed additive according to claim 17 or 18, c h a r a c t e r i z e d in that said oilseeds are soybeans and that said feed or feed additive contains soya oil.

21. Animal feed or feed additive according to claim 17, 18 or 19, c h a r a c t e r i z e d in that it is free of any insoluble portions of said oilseeds.

22. Animal feed or feed additive according to claim 17, c h a r a c t e r i z e d in that it contains said degradation products absorbed in a water insoluble solid fraction of crushed dehulled soya beans.

23. Fish feed c h a r a c t e r i z e d in that it consists essentially of the degradation products of oilseed proteins obtained by an aqueous proteolytic degradation of the substantially non-denatured proteins and proteinaceous antinutritional factors of raw oilseeds.

24. Fish feed according to claim 23, wherein said proteins are soy proteins which have been degraded to substantially uniform small fragments so that at least 80% thereof, preferably at least 90% thereof have a size less than 10 kD.

25. Fish feed according to claim 34, which additionally contains soya oil.

26. The use of the degradation products of oilseed proteins obtained by an aqueous proteolytic degradation of the substantially non-denatured proteins and proteinaceous anti-nutritional factors of raw oilseeds for the production of a nutritional product.

27. The use of claim 26, wherein said nutritional product is a fish feed.

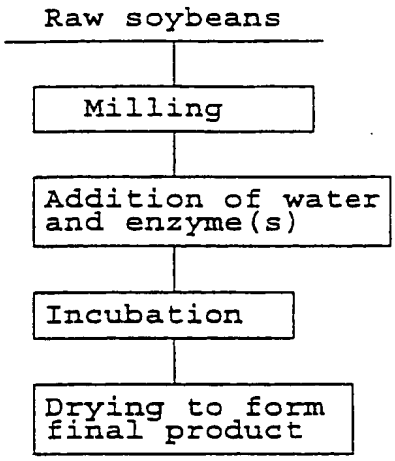
Raw soybeans
Milling
Addition of water and enzyme(s)
Incubation
Drying to form final product

FIG. 1

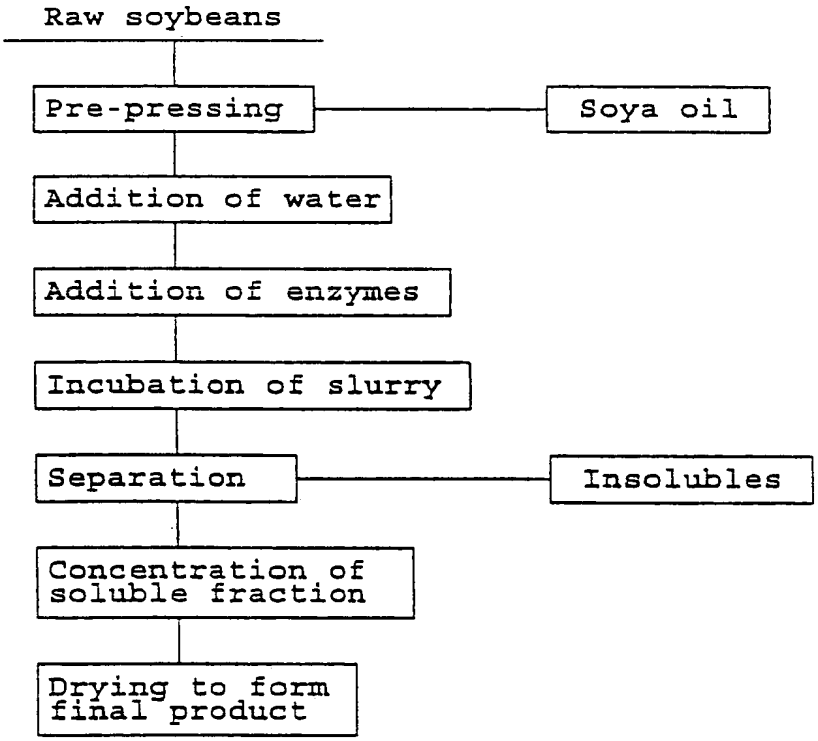
Raw soybeans
Pre-pressing
Soya oil
Addition of water
Addition of enzymes
Incubation of slurry
Separation
Insolubles
Concentration of soluble fraction
Drying to form final product

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 98/00508

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A23J 3/16, A23J 3/34, A23K 1/14, A23L 1/305
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A23J, A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0138544 A2 (GENENTECH, INC.), 24 April 1985 (24.04.85), See especially page 9, line 6 - line 27	1-27
A	WO 9113554 A1 (NOVO NORDISK A/S), 19 Sept 1991 (19.09.91), See especially page 8, line 8 - line 15	12,18,24
A	WO 9529598 A1 (GIST-BROCADES B.V.), 9 November 1995 (09.11.95)	1-27

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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"E" erlier document but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 Sept 1998	28 -09- 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Anneli Jönsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No.
PCT/FI 98/00508

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